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(54) Title: TASQUINIMOD OR A PHARMACEUTICALLY ACCEPTABLE SALT THEREOF FOR USE IN THE TREATMENT OF MYELOYDYSPLASTIC SYNDROME

(57) Abstract: Tasquinimod, or a pharmaceutically acceptable salt thereof, for use in the treatment of myelodysplastic syndrome (MDS).

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TASQUINIMOD OR A PHARMACEUTICALLY ACCEPTABLE SALT THEREOF FOR USE
IN THE TREATMENT OF MYELOYDYSPLASTIC SYNDROME

FIELD OF THE INVENTION

- 5 The present invention relates to a novel use of the quinoline derivative tasquinimod. More particularly, the invention relates to tasquinimod or a pharmaceutically acceptable salt thereof for use in the treatment of myelodysplastic syndrome.

BACKGROUND OF THE INVENTION

- 10 Tasquinimod and a method for its preparation were described in International Applications No. PCT/SE99/00676, published as WO 99/55678, and No. PCT/SE99/01270, published as WO 00/03991, which applications also disclosed the utility of tasquinimod and some other quinoline carboxamides for the treatment of diseases resulting from autoimmunity, such as multiple sclerosis, insulin-dependent diabetes mellitus, systemic lupus erythematosus,
15 rheumatoid arthritis, inflammatory bowel disease and psoriasis and, furthermore, diseases where pathologic inflammation plays a major role, such as asthma, atherosclerosis, stroke and Alzheimer's disease.

- Processes for preparing tasquinimod are also disclosed in International Application No.
20 PCT/SE2003/000780, published as WO 03/106424 and International Application No. PCT/EP2011/061490, published as WO 2012/004338. A deuterated form of tasquinimod was described in International Application No. PCT/EP2012/061798, published as WO 2012/175541.

- 25 The use of various quinoline carboxamides for the treatment of cancer, more particularly solid cancers, such as prostate cancer and breast cancer, is disclosed in International Application No. PCT/SE00/02055, published as WO 01/30758. It has been found that some of these compounds bind to and inhibit the interactions of an immunomodulatory protein (S100A9), which protein promotes tumor development, influences suppressive and pro-
30 angiogenic cells in the tumor microenvironment and participates in the establishment of pre-metastatic niches.

- International Application No. PCT/EP2015/075769, published as WO 2016/078921, discloses tasquinimod for use in the treatment of leukemia including acute lymphoblastic leukemia,
35 acute myeloid leukemia, chronic lymphocytic leukemia and chronic myeloid leukemia.

International Application No. PCT/EP2015/071391, published as WO 2016/042112, discloses tasquinimod for use in the treatment of multiple myeloma.

International Application No. PCT/EP2016/053288, published as WO 2016/146329, discloses tasquinimod for use in combination with a PD-1 and/or PD-L1 inhibitor in the treatment of cancer, in particular bladder cancer.

Myelodysplastic syndromes (MDS) are a heterogeneous spectrum of chronic myeloid neoplasia or clonal hematopoietic stem cell disorders, associated with ineffective hematopoiesis manifesting as symptomatic cytopenias, morphologic dysplasia and a substantial risk of progression to acute myeloid leukemia.

MDS are one of the most common haematological neoplasms in the elderly population with a median age of 65-70 years; however, disease can occur at any age. While no apparent risk factor is present in primary MDS, DNA damage by previous chemo- or radiation therapy is a well-known risk factor for secondary MDS. Further, MDS in the paediatric population might arise secondary to inherited bone marrow failures (for example Fanconi anemia, or dyskeratosis congenital).

Multiple biological processes govern hematopoietic progenitor proliferation and differentiation into mature blood cells. Molecular alterations in hematopoietic stem cells that disrupt any of these processes are integral to the pathogenesis of MDS. About 80-90% of MDS patients have a somatic mutation in their hematopoietic stem cells. The type and incidence of somatic mutations contribute to dysfunctional signalling pathways in MDS and are associated with disease prognosis and responsiveness to certain drugs. Besides molecular alterations of the hematopoietic stem cell, the surrounding bone marrow niche plays a central role in MDS pathogenesis. Aberrant inflammatory signalling, including the activation of the NLRP3 inflammasome, may facilitate the selection, maintenance and leukemic evolution of the malignant MDS clone.

MDS patients can be asymptomatic at diagnosis, however, up to 80% suffer from anemia. Patients are primarily affected by cytopenia-related symptoms, such as fatigue and weakness due to anemia, neutropenia-related infections or bleeding due to thrombocytopenia. About 1/3 of the MDS patients will progress to acute myeloid leukemia.

The revised International Prognostic Scoring System for MDS (IPSS-R) is the most common used prognostication system to determine disease prognosis. The model demonstrates 5 risk

categories according to the grade of cytopenia, marrow blast percentage and cytogenetic subgroup.

Even though MDS is one of the most common hematologic neoplasms in the elderly population, only limited treatment options are available. Besides supportive care, only five drugs are approved in Europe (erythropoietin, luspatercept, the iron chelator deferasirox, the immunomodulatory drug lenalidomide, and the hypomethylating agent azacitidine). The only curative treatment is allogeneic hematopoietic stem cell transplantation, which is however not feasible in many elderly patients.

Supportive care includes transfusion therapy, treatment with erythropoiesis-stimulating agents, and antibiotic therapy. Transfusion therapy (blood transfusion) is a method of giving red blood cells, white blood cells, or platelets to replace blood cells destroyed by disease or treatment. Frequent red blood cell transfusions lead to iron overload and accompanying organ toxicities with poor clinical outcome. Iron chelation therapy with deferasirox might improve patient's outcome. Erythropoiesis-stimulating agents (ESAs) are given to increase the number of mature red blood cells made by the body and to lessen the effects of anemia. Sometimes granulocyte colony-stimulating factor (G-CSF) is given with ESAs to help the treatment work better. Finally, antibiotic therapy may be used to fight infection.

Drug therapy includes treatment with e.g. lenalidomide, which is only approved in patients with myelodysplastic syndrome associated with an isolated del(5q) chromosome abnormality who need frequent red blood cell transfusions. Patients with transfusion-dependent MDS with ring sideroblasts, refractory or unlikely to respond to ESA, can use luspatercept. The hypomethylating agent azacitidine may be used to treat myelodysplastic syndromes in advanced disease stages to slow the progression of myelodysplastic syndromes to acute myeloid leukemia. Usually, classical chemotherapy is only used before stem cell transplantation in patients with higher blast count to reduce tumor cell burden.

During allogeneic stem cell transplantation, stem cells (immature blood cells) are collected from the blood or bone marrow of a healthy donor and reinfused to the MDS patient after completion of conditioning therapy. The donor hematopoietic stem cells will replace the patient's original bone marrow and restore the body's blood cells (termed engraftment).

SUMMARY OF THE INVENTION

A first aspect is tasquinimod or a pharmaceutically acceptable salt thereof for use in the treatment of myelodysplastic syndrome.

A further aspect is the use of tasquinimod or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of myelodysplastic syndrome.

- 5 Still a further aspect is a pharmaceutical formulation containing a therapeutically effective amount of tasquinimod or a pharmaceutically acceptable salt thereof, for the treatment of myelodysplastic syndrome.

A further aspect is a method for the treatment of myelodysplastic syndrome in a mammal in
10 need of such treatment, by administration of a therapeutically effective amount of tasquinimod or a pharmaceutically acceptable salt thereof to the mammal.

Further aspects and embodiments thereof will become apparent from the following description and claims.

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BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a graph representing the concentration of S100A9 (in pg/ml) in bone marrow obtained from healthy patients (control), and patients with low risk MDS (LR-MDS) and high risk MDS (HR-MDS), respectively.

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FIGURE 2 shows Western blots of phosphorylated IRAK1 (80 kDa), NF- κ B-p65 (65 kDa) and gasdermin (50 kDa) in MDS MSCs following treatment with S100A9 or with S100A9 and tasquinimod, for 48h. GAPDH (38 kDa) served as reference protein. Lane 1 = untreated control; lane 2 = treated with S100A9; lane 3 = treated with S100A9/tasquinimod.

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FIGURE 3 is a bar chart representing the mRNA expression levels of IL-1 β , IL-18, Casp1 and PD-L1 by real-time PCR of MDS MSCs after 48h treatment with tasquinimod only (TASQ), with S100A9 only (S100A9), or with both S100A9 and tasquinimod (S100A9+TASQ). Amplicons for IL-1 β , IL-18, Casp1 and PD-L1 were normalized to
30 endogenous GAPDH expression and the untreated MSCs were set to 1 (=control).

FIGURE 4 shows a Western blot of PD-L1 in healthy and CMML MSCs following treatment with S100A9 and tasquinimod for 48h. Lane 1 = untreated control; lane 2 = treated with tasquinimod; lane 3 = treated with S100A9; lane 3 = treated with S100A9 and tasquinimod.

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FIGURE 5 shows a Western blot of phosphorylated IRAK1 (80 kDa), NF- κ B-p65 (65 kDa) and gasdermin (50 kDa) in MDS MSCs following treatment with TNF- α and tasquinimod for

48h. GAPDH (38 kDa) served as reference protein. Lane 1 = treated with TNF- α ; lane 2 = treated with TNF- α and tasquinimod.

FIGURE 6a is a bar chart showing quantification of CAF-C when co-culturing MDS MSC with hematopoietic progenitor cells, after one, two, three and four weeks without the presence of either S100A9 or tasquinimod ("without"), in the presence of tasquinimod (TASQ), in the presence of S100A9 (S100A9), and in the presence of both s100A9 and tasquinimod (S100A9+TASQ).

FIGURE 6b is a bar chart showing the number of colonies/300 cells in CFU assays performed using MDS MSC cells cocultured with hematopoietic progenitor cells, harvested after one week of co-culture in the presence of S100A9 or in the presence of both S100A9 and tasquinimod or in the absence of both S100A9 and tasquinimod ("control"), with 300 cells being plated in enriched methylcellulose medium with recombinant cytokines (MethoCult™ H4435, STEMCELL Technologies). Colonies were counted after 2 weeks and classified under a microscope or with the STEMvision™ system (STEMCELL Technologies).

FIGURE 6c is a bar chart showing the number of colonies/300 cells in CFU assays performed using MDS MSC cells and hematopoietic progenitor cells, harvested after one week of co-culture in the presence ("TASQ") or absence ("without") of tasquinimod, with 300 cells being plated in enriched methylcellulose medium with recombinant cytokines (MethoCult™ H4435, STEMCELL Technologies). Colonies were counted after 2 weeks and classified under a microscope or with the STEMvision™ system (STEMCELL Technologies).

DETAILED DESCRIPTION

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains.

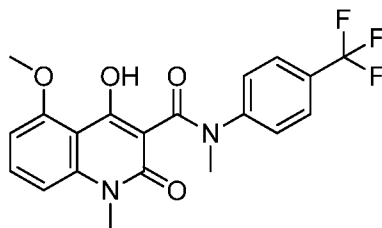
The abbreviations and acronyms used in this text are considered as well-known to the person of ordinary skill in the field art, but for the sake of clarity the meaning of some of these is indicated herein below:

ALP	alkaline phosphatase
α SMA	alpha-smooth muscle actin
BCA	bicinchoninic acid assay
BFU-E	burst forming unit-erythroid

	BM	bone marrow
	CAF-C	cobblestone area forming-cell
	cDNA	complementary DNA
	CFU	colony forming unit
5	CFU-E	colony forming unit-erythroid
	CFU-GEMM	colony forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte
	CFU-GM	colony forming unit-granulocyte/macrophage
	CMML	chronic myelomonocytic leukemia
	Cy	cyanine
10	DAPI	4',6-diamidino-2-phenylindole
	DMEM	Dulbecco's Modified Eagle Medium
	dT	deoxythymidine
	ECAR	extracellular acidification rate
	ECL	enhanced chemiluminescence
15	EDTA	ethylenediaminetetraacetic acid
	ELISA	enzyme-linked immunosorbent assay
	FCCP	carbonyl cyanide-p-trifluoromethoxyphenylhydrazone
	FCS	fetal calf serum
	FLT3-L	fms-like tyrosine kinase 3 ligand
20	GAPDH	glyceraldehyde 3-phosphate dehydrogenase
	HRP	horseradish peroxidase
	HSA	human serum albumin
	HSC(s)	hematopoietic stem cell(s)
	IgG	immunoglobulin G
25	IL	interleukin
	IRAK1	Interleukin-1 receptor-associated kinase 1
	MDS	myelodysplastic syndrome
	MDS-RS	myelodysplastic syndrome with ring sideroblasts
	MSC(s)	mesenchymal stem cell(s)
30	NF- κ B	nuclear factor kappa-light-chain-enhancer of activated B cells
	OCR	oxygen consumption rate
	PBMC	Peripheral blood mononuclear cell
	PBS	phosphate buffered saline
	PD-1	programmed cell death protein 1
35	PD-L1	programmed death-ligand 1
	RIPA	radioimmunoprecipitation assay
	RNA	ribonucleic acid

ROS	reactive oxygen species
ROX	carboxy-X-rhodamine
RT-PCR	real-time polymerase chain reaction
SCF	stem cell factor
5 SDS	sodium dodecyl sulfate
TLR4	toll like receptor 4
TNF- α	tumor necrosis factor alpha

10 The compound tasquinimod, or 4-hydroxy-5-methoxy-N,1-dimethyl-2-oxo-N-[4-(trifluoromethyl)phenyl]-1,2-dihydroquinoline-3-carboxamide, has the structural formula



Optional" or "optionally" means that the subsequently described event or circumstance may but need not occur, and that the description includes instances where the event or
15 circumstance occurs and instances in which it does not.

"Pharmaceutically acceptable" means that which is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and neither biologically nor otherwise undesirable and includes that which is acceptable for veterinary use as well as human
20 pharmaceutical use.

Examples of pharmaceutically acceptable salts comprise salts with (as counter ion) an alkali metal ion, e.g. Li⁺, Na⁺ or K⁺, or salts with an alkaline earth metal ion, e.g. Mg²⁺ or Ca²⁺, or salts with any other pharmaceutically acceptable metal ion, e.g. Zn²⁺ or Al³⁺; or
25 pharmaceutically acceptable salts formed with organic bases, such as diethanolamine, ethanolamine, N-methylglucamine, triethanolamine or tromethamine.

"Therapeutically effective amount" means an amount of tasquinimod or a pharmaceutically salt thereof, that, when administered to a subject for treating a condition (here: MDS), is
30 sufficient to effect such treatment for the condition. The "therapeutically effective amount" will vary depending on e.g. the age and relative health of the treated subject, the state of progression of the condition, the route and form of administration, the possible additional use of other drugs, e.g. in a combination therapy, etc. In some embodiments, an "effective

amount" provides treatment effect (e.g., treats, prevents, inhibits, relieves, promotes, improves, increases, reduces, and the like) as measured by a statistically significant change in one or more indications, symptoms, signs, diagnostic tests, vital signs, and the like. In further embodiments, an "effective amount" suppresses, manages, or prevents a condition as measured by a lack of a statistically significant change in one or more indications, symptoms, signs, diagnostic tests, vital signs, and the like.

As used herein the terms "treatment" or "treating" is an approach for obtaining beneficial or desired results including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms of the treated condition, diminishment of extent of the condition, stabilization (i.e., not worsening) of the state of the condition, preventing spread of the condition, delay or slowing of progression of the condition, amelioration or palliation of the condition, and remission (whether partial or total), whether detectable or undetectable. The term can also mean prolonging survival as compared to expected survival without the treatment.

MDS patients are primarily affected by cytopenia, such as anemia, thrombocytopenia, neutropenia, bicytopenia and pancytopenia and their related symptoms, such as fatigue, anemia, weakness, easy bruising or bleeding, fever, bone pain, shortness of breath, and frequent infections.

The term "mammal" refers to a human or any mammalian animal, e.g. a primate, a farm animal, a pet animal, or a laboratory animal. Preferably, the mammal is a human.

The mammal (e.g. human) subject that may suitably be treated according to the present invention may be one suffering from MDS, or one at (increased) risk of developing MDS.

As used herein, "MDS" refers to a group of acquired hematopoietic disorders characterized by peripheral cytopenias and a normocellular or hypercellular bone marrow. Subtypes of MDS include MDS with unilineage (single lineage) dysplasia, MDS with multilineage dysplasia, MDS with ring sideroblasts (MDS-RS), MDS associated with isolated del chromosome abnormality, such as MDS with isolated del(5q), MDS with excess blasts, and MDS, unclassifiable.

The WHO has classified the various forms of MDS into different subtypes, depending on criteria such as the percentage of myeloblasts in the bone marrow, the presence of abnormal red blood cell precursors (ringed sideroblasts) in the bone marrow, the number of abnormal

cell types known as dysplastic lineages in the bone marrow, and the genetic profile of the bone marrow cells, as follows:

MDS with single-lineage dysplasia (MDS-SLD) – 1 or 2 blood cytopenias; in bone marrow, dysplasia in $\geq 10\%$ of one cell line, $< 5\%$ blasts

MDS with multilineage dysplasia (MDS-MLD) – 1-3 blood cytopenias, $< 1 \times 10^9/L$ monocytes; in bone marrow, dysplasia in $\geq 10\%$ of cells in ≥ 2 hematopoietic lineages $< 15\%$ ring sideroblasts (or $< 5\%$ ring sideroblasts if *SF3B1* mutation present) $< 5\%$ blasts

MDS with ring sideroblasts (MDS-RS) – anemia, no blasts; in bone marrow, $\geq 15\%$ of erythroid precursors with ring sideroblasts or $\geq 5\%$ ring sideroblasts if *SF3B1* mutation is present

MDS with isolated del(5q) – anemia, platelets normal or decreased; in bone marrow, unilineage erythroid dysplasia, isolated del(5q), $< 5\%$ blasts $\pm$ one other abnormality except - 7/del(7q)

MDS with excess blasts (MDS-EB) – 1-3 blood cytopenias, 0-3 dysplastic bone marrow lineages, and 5-9% blasts in bone marrow or 2-4% blasts in blood (MDS-EB1) or 10-19% blasts in bone marrow or 5-19% blasts in blood (MDS-EB2)

Unclassifiable MDS – cytopenias, $\pm 1\%$ blasts on at least 2 occasions; in bone marrow, single-lineage dysplasia or no dysplasia but characteristic MDS cytogenetics, $< 5\%$ blasts.

The WHO classification also includes the provisional category of refractory cytopenia of childhood, with cytopenias and $< 2\%$ blasts in peripheral blood and, in bone marrow, dysplasia in 1–3 lineages and $< 5\%$ blasts.

Additionally, according to the WHO classification, CMML (CMML-0, CMML-1, CMML-2) and MDS/MPN-RS-T are classical MDS/MPN overlap syndromes.

The classification developed by the WHO is used herein to define the various subtypes of MDS, but unless otherwise specified or apparent from the context, the term MDS as used herein is considered to include any subtype of MDS, e.g. any of the above indicated subtypes, also including CMML. In some embodiments, however, the MDS more particularly is MDS with single-lineage dysplasia as defined herein above. In some embodiments, the

MDS more particularly is MDS with multilineage dysplasia (MDS-MLD) as defined herein above. In some embodiments, the MDS more particularly is MDS with ring sideroblasts (MDS-RS) as defined herein above. In some embodiments, the MDS more particularly is MDS with isolated del(5q) as defined herein above. In some embodiments, the MDS more particularly is MDS with excess blasts (MDS-EB) as defined herein above. In some embodiments, the MDS more particularly is unclassifiable MDS as defined herein above. In some embodiments, the MDS more particularly is refractory cytopenia of childhood, as defined herein above. In some embodiments, the MDS more particularly includes CMML. In some further embodiments, the MDS does not include CMML.

In addition to the diagnostic classification system mentioned above, a prognostic system, called Revised International Prognostic Scoring System (IPSS-R) has been developed, according to which MDS can be classified as very low risk, low risk, intermediate risk or high risk or very high risk, cf. guidance published by Greenberg, Tuechler, Schanz et al., Revised International Prognostic Scoring System (IPSS-R for Myelodysplastic Syndrome, Blood 120: 2454, 2012), and Schanz J et al, (J Clin Oncology 2012; 30:820). The system uses hemoglobin count (g/dl), absolute neutrophil count ($\times 10^9/L$), platelets ($\times 10^9/L$), bone marrow blast (percent), and cytogenetic category of the MDS. The cytogenetic category is defined as very good, good, intermediate, poor, or very poor, based on the presence of certain cytogenetic abnormalities, as represented in Table 1.

Table 1

CYTOGENETIC PROGNOSTIC SUBGROUPS	CYTOGENETIC ABNORMALITIES
Very good	-Y, del(11q)
Good	Normal, del(5q), del(12p), del(20q), double including del(5q)
Intermediate	del(7q), +8, +19, i(17q), any other single or double independent clones
Poor	-7, inv(3)/t(3q)/del(3q), double including -7/del(7q), Complex: 3 abnormalities
Very poor	Complex: >3 abnormalities

The cytogenetic prognostic subgroup, and the determined value of each of the above-mentioned criteria (hemoglobin count (g/dl), absolute neutrophil count (ANC) ($\times 10^9/L$), platelets ($\times 10^9/L$), bone marrow blast (percent)) of the patient are scored as shown in Table 2

Table 2

PROGNOSTIC VARIABLE	0	0.5	1	1.5	2	3	4
Cytogenetics	Very Good		Good		Intermediate	Poor	Very Poor
BM Blast (%)	≤2		>2-<5%		5-10%	>10%	
Hemoglobin (g/dl)	≥10		8-<10	<8			
Platelets x10 ⁹ /L	≥100	50-<100	<50				
ANC x10 ⁹ /L	≥0.8	<0.8					

The total risk score obtained is used to classify the MDS into an IPSS-R Prognostic Risk Category, as shown in Table 3

Table 3

RISK CATEGORY	RISK SCORE
Very Low	≤1.5
Low	>1.5 - 3
Intermediate	>3 - 4.5
High	>4.5 - 6
Very High	>6

Finally, based on data from over 7000 patients, the IPSS-R has associated each prognostic risk category with a median survival time in years and a median time to 25 % AML evolution, as indicated in Table 4.

Table 4

Risk category	Very Low	Low	Intermediate	High	Very High
Survival*	8.8 years	5.3 years	3.0 years	1.6 years	0.8 year
AML/25 %**	NR	10.8 years	3.2 years	1.4 years	0.7 year

* medians, ** median time to 25 % AML evolution

In some embodiments, the indication MDS includes various forms of anemia, such as refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess

blasts, refractory anemia with excess blasts in transformation and chronic myelomonocytic leukemia. In some embodiments, the indication MDS excludes MDS that has developed into leukemia. In some embodiments, the patient does not have a leukemia.

- 5 In some embodiments, tasquinimod is for use in the treatment of an MDS that has been classified into a risk score according to the Revised International Prognostic Scoring System (IPSS-R) for Myelodysplastic Syndromes as mentioned herein.

10 Unless otherwise specified herein, the MDS may be within any of the above identified risk categories, as determined by use of the above-mentioned Revised International Prognostic Scoring System (IPSS-R).

15 In some embodiments, the MDS belongs to the risk category defined as very low (having a risk score of lower than or equal to 1.5), low (having a risk score of higher than 1.5 and lower than or equal to 3), or intermediate (having a risk score of higher than 3 and lower than or equal to 4.5). In some of these embodiments, the MDS belongs to the risk category defined as low or intermediate, e.g. as intermediate. In some further embodiments, the MDS belongs to the risk category defined as very low or low, e.g. as low. In still further embodiments, the MDS belongs to the risk category defined as very low.

20 In some further embodiments, the MDS belongs to the risk category defined as intermediate, high (having a risk score of higher than 4.5 and lower than or equal to 6) or very high (having a risk score of higher than 6). In some of these embodiments, the MDS belongs to the risk category defined as intermediate or high, e.g. as high. In some further embodiments, the MDS belongs to the risk category defined as high or very high, e.g. as very high.

Anemia is an important, not to say predominant, cause of morbidity and quality of life impairment in MDS patients, in particular those with lower risk MDS (e.g., very low risk, low risk, or intermediate risk MDS). Therefore, in some embodiments, tasquinimod is used in the treatment of anemia in a patient having a lower risk MDS. In some embodiments, tasquinimod is used in the treatment of anemia in connection with MDS. In some embodiments, tasquinimod is used in the treatment of MDS associated with anemia as a symptom thereof. In some embodiments, the anemia is selected from refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation and chronic myelomonocytic leukemia. In some embodiments, the anemia is refractory anemia. In some embodiments, the anemia is refractory anemia with ringed sideroblasts. In some embodiments, the anemia is refractory

anemia with excess blasts. In some embodiments, the anemia is refractory anemia with excess blasts in transformation and chronic myelomonocytic leukemia.

In some embodiments, tasquinimod is for use in the treatment of an MDS in a patient for which at least one clinical parameter has been determined, selected from hemoglobin count, absolute neutrophil count, platelet count, bone marrow blast (percent), and cytogenetic abnormalities.

For example, in some embodiments, the MDS belongs to the cytogenetic prognostic subgroup termed "very good", as defined in the Table 1. In some other embodiments, the MDS belongs to the cytogenetic prognostic subgroup termed "good", as defined in Table 1. In still other embodiments, the MDS belongs to the cytogenetic prognostic subgroup termed "intermediate", as defined in Table 1. In still other embodiments, the MDS belongs to the cytogenetic prognostic subgroup termed "poor", as defined in Table 1. In still other embodiments, the MDS belongs to the cytogenetic prognostic subgroup termed "very poor", as defined in Table 1.

In some embodiments, the MDS patient has a BM blast of less than 2%. In some embodiments, the MDS patient has a BM blast of higher than or equal to 2% and lower than 5%. In some embodiments, the MDS patient has a BM blast of higher than or equal to 5% and lower than 10%. In some embodiments, the MDS patient has a BM blast of higher than 10%.

In some embodiments, the MDS patient has a hemoglobin level of higher than or equal to 10 g/dl. In some embodiments, the MDS patient has a hemoglobin level of higher than or equal to 8 g/dl and lower than 10 g/dl. In some embodiments, the MDS patient has a hemoglobin level of lower than 8 g/dl.

In some embodiments, the MDS patient has a platelet count of higher than or equal to $100 \times 10^9/L$. In some embodiments, the MDS patient has a platelet count of higher than or equal to $50 \times 10^9/L$ and lower than $100 \times 10^9/L$. In some embodiments, the MDS patient has a platelet count of lower than $50 \times 10^9/L$.

In some embodiments, the MDS patient has an absolute neutrophil count of higher than or equal to $0.8 \times 10^9/L$. In some embodiments, the MDS patient has an absolute neutrophil count of lower than $0.8 \times 10^9/L$.

In some embodiments tasquinimod is provided for use in a treatment method that comprises improving one or more hematologic parameters in a mammal suffering from or at risk of developing MDS (e.g. a mammal patient having MDS). Such improvement of a hematological parameter may be selected from decreasing myoblasts, increasing hemoglobin, increasing platelets, increasing neutrophils, decreasing hepcidin, reducing units of red blood cell transfused, reducing frequency of transfusion, and reducing transfusion dependence.

As mentioned herein above, tasquinimod, pharmaceutically acceptable salts thereof, deuterated forms thereof, crystalline salts thereof, and pharmaceutical compositions containing the compounds and their salts, as well as methods for preparing such compounds, their salts, deuterated forms and pharmaceutical compositions containing the compounds and their salts have been described in WO 99/55678, WO 00/03991, WO 03/106424, WO 2005/074899, WO 2012/004338 and WO 2012/175541 (*vide supra*), which documents are hereby incorporated by reference in their entireties into the present application.

In some embodiments, any reference to tasquinimod also encompasses the deuterated form of thereof. As mentioned herein above, a deuterated form of tasquinimod and a method for preparing such form are described in WO 2012/175541. In some embodiments, thus, tasquinimod has a deuterium enrichment in the carboxamide-N methyl group of at least 70%, more preferably at least 90%.

In some embodiments, tasquinimod is non-deuterated, having a deuterium content corresponding to the natural abundance of deuterium.

The present invention includes tasquinimod or a pharmaceutically acceptable salt thereof, formulated in a pharmaceutical composition, optionally together with a pharmaceutically acceptable excipient, e.g. a carrier, for use in the treatment of MDS.

In some embodiments, use is made of a pharmaceutically acceptable salt of tasquinimod.

The pharmaceutical composition may be suitable for enteral administration, such as rectal or oral administration, or for parenteral administration to a mammal (especially a human), and comprises a therapeutically effective amount of tasquinimod or a pharmaceutically acceptable salt thereof, as active ingredient, optionally in association with a pharmaceutically acceptable excipient, e.g. a pharmaceutically acceptable carrier. The therapeutically effective amount of the active ingredient is as defined herein above and depends e.g. on the species

of mammal, the body weight, the age, the individual condition, individual pharmacokinetic data, and the mode of administration.

For enteral, e.g. oral, administration, tasquinimod may be formulated in a wide variety of dosage forms. The pharmaceutically acceptable carriers may be either solid or liquid. Solid form preparations include powders, tablets, pills, lozenges, capsules, cachets, suppositories, and dispersible granules. A solid carrier may be one or more substances which may also act as diluents, flavouring agents, solubilizers, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. In powders, the carrier generally is a finely divided solid which is a mixture with the finely divided active component. In tablets, the active component generally is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired. Suitable carriers include but are not limited to magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatine, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like.

Other forms suitable for oral administration include liquid form preparations including emulsions, syrups, elixirs, aqueous solutions, aqueous suspensions, or solid form preparations which are intended to be converted shortly before use to liquid form preparations. Emulsions may be prepared in solutions, for example, in aqueous propylene glycol solutions or may contain emulsifying agents, for example, such as lecithin, sorbitan monooleate, or acacia. Aqueous solutions can be prepared by dissolving the active component in water and adding suitable colorants, flavors, stabilizers, and thickening agents. Aqueous suspensions can be prepared by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well-known suspending agents. Solid form preparations include solutions, suspensions, and emulsions, and may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

Exemplary compositions for rectal administration include suppositories which can contain, for example, a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperatures, but liquefy and/or dissolve in the rectal cavity to release the drug.

Tasquinimod also may be administered parenterally, e.g. by injection or infusion, e.g. by intravenous, intraarterial, intraosseous, intramuscular, intracerebral, intracerebroventricular,

intrasynovial, intrasternal, intrathecal, intralesional, intracranial, intratumoral, intracutaneous and subcutaneous injection or infusion. Thus, for parenteral administration, the pharmaceutical compositions may be in the form of a sterile injectable or infusible preparation, for example, as a sterile aqueous or oleaginous suspension. This suspension
5 may be formulated according to techniques known in the art using suitable dispersing or wetting agents (e.g., Tween® 80), and suspending agents. The sterile injectable or infusible preparation may also be a sterile injectable or infusible solution or suspension in a non-toxic parenterally acceptable diluent or solvent. For example, the pharmaceutical composition may be a solution in 1,3-butanediol. Other examples of acceptable vehicles and solvents that may
10 be employed in the compositions of the present invention include, but are not limited to, mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as
15 are natural pharmaceutically acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant.

Solutions for parenteral use also may contain suitable stabilizing agents, and if necessary,
20 buffer substances. Suitable stabilizing agents include antioxidizing agents, such as sodium bisulfate, sodium sulfite or ascorbic acid, either alone or combined, citric acid and its salts and sodium EDTA. Parenteral solutions may also contain preservatives, such as benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol.

25 Conventional procedures for the selection and preparation of suitable pharmaceutical formulations are described, for example, in "Pharmaceutics - The Science of Dosage Form Design", M.B. Aulton, Churchill Livingstone, 2nd ed. 2002 (ISBN 0443055173, 9780443055171). Suitable pharmaceutical excipients, e.g. carriers, and methods of preparing pharmaceutical dosage forms also are described in Remington's Pharmaceutical
30 Sciences, Mack Publishing Company, a standard reference text in art of drug formulation.

The pharmaceutical compositions (which may also be referred to herein as pharmaceutical formulations, or medicaments) will comprise a therapeutically effective amount of tasquinimod or a pharmaceutically acceptable salt thereof, e.g. they may comprise from
35 approximately 1 % to approximately 95%, preferably from approximately 20% to approximately 90% of tasquinimod or the salt thereof, together with at least one pharmaceutically acceptable excipient. In general, tasquinimod or its salt will be administered

in a therapeutically effective amount by any of the accepted modes of administration for agents that serve similar utilities.

5 While e.g. injection or rectal administration of tasquinimod or its salt may be contemplated if necessary, oral administration generally is considered the most convenient.

10 The dosage level and frequency will generally be as determined by the treating physician, with due regard to factors such as and the sex, age, corporal weight and relative health of the treated subject, the severity of the MDS, the selected route and form of administration, the additional use of other drugs, e.g. in a combination therapy.

15 Generally, a daily dosage ranging from a minimum of 0.001 mg/kg body weight, or 0.002 mg/kg body weight or 0.005 mg/kg body weight or 0.01 mg/kg body weight, to a maximum of 0.2 mg/kg body weight, or 0.1 mg/kg body weight, or 0.05 mg/kg body weight, or 0.02 mg/kg body weight is contemplated.

In one embodiment, tasquinimod is administered in an amount of 0.05 to 0.15 mg/day, or 0.08 to 0.1 mg/day, e.g. 0.1 mg/day.

20 In one embodiment, tasquinimod is administered in an amount of 0.1 to 0.3 mg/day, or 0.15 to 0.25 mg/day, e.g. 0.2 mg/day.

25 In one embodiment, tasquinimod is administered in an amount of 0.1 to 1 mg/day, or 0.2 to 0.8 mg/day, e.g. 0.5 mg/day.

In one embodiment, tasquinimod is administered in an amount of 0.2 to 1.5 mg/day, or 0.4 to 1.2 mg/day, e.g. 0.8 mg/day.

30 In one embodiment, tasquinimod is administered in an amount of 0.5 to 2 mg/day, or 0.8 to 1.2 mg/day, e.g. 1 mg/day.

In one embodiment, tasquinimod is administered in an amount of 0.8 to 3 mg/day, or 1 to 2.5 mg/day, e.g. 2 mg/day.

35 In one embodiment, tasquinimod is administered in an amount of 1 to 6 mg/day, or 2 to 4 mg/day, e.g. 3 mg/day.

In some embodiments, the dosage may be gradually adjusted to reach optimal results, so-called dosage titration. For example, dosage titration may comprise starting with a low daily dosage of e.g. 0.25 mg and maintaining this dose level for a period of 1 or 2 weeks. In case no significant side effects are encountered that may contraindicate raising the dose, the level
5 may then be increased, e.g. to 0.5 mg/day for 1 or 2 weeks, after which period another increase may be contemplated, to reach a daily dosage of 1 mg, and so on. In such a method, if any significant side effects occur after an incremental increase of the dosage, the dosage may again be reduced to a previous level.

- 10 Side effects that may occur include those that may generally be encountered in this type of treatment, e.g. gastrointestinal problems, tiredness, and flu-like syndrome, considered to be related to dosage.

Tasquinimod preferably is administrated on a daily basis, e.g. 1-3 times a day, or 1-2 times a
15 day, such as once daily. In some embodiments, the drug is administrated on a less frequent basis, e.g. every two days, once a week etc. It should also be noted that if a pharmaceutically acceptable salt of tasquinimod is administered, an equivalent dosage would be one resulting in the indicated dosage of the compound in non-salt form.

- 20 In its broadest sense, the present invention relates to tasquinimod or a pharmaceutically acceptable salt thereof, for use in the treatment of MDS. In some embodiments, the MDS is selected from any of the subtypes mentioned herein above.

BIOLOGICAL ASSAYS

25 Materials and methods

Patients

Heparinized BM samples were obtained from untreated MDS patients (low-risk MDS, low-risk MDS with del5q, high-risk MDS, and CMML) during standard diagnostic aspiration, and from hematological healthy donors undergoing hip replacement surgery. The samples were
30 analyzed for level of S100A9 using the Human DuoSet ELISA kit (R&D Systems) according to manufacturer's instructions.

Cell culture

BM mononuclear cells were isolated by percoll gradient centrifugation and MSCs were grown
35 as adherent monolayer in DMEM/10% FCS. Passages 2–5 were used for the experiments.

To mimic inflammatory conditions, *in-vitro* cultured MSCs were treated with recombinant S100A8, S100A9 or the S100A8/9 heterodimer (each 1.5 µg/ml) or TNF-α (10 ng/ml) in the presence or absence of tasquinimod (10 µM) for different time periods depending on the subsequent experiments. Hematopoietic stem and progenitor cells (HSPCs) for cocultures were isolated using CD34 antibody-conjugated magnetic beads, according to the manufacturer's instructions (Miltenyi Biotec) and were added in CellGro medium (CellGenix) containing stem cell factor (SCF), FLT3-L and IL-3 (10 ng/ml each).

Peripheral blood mononuclear cells (PBMCs) were prepared by Ficoll-Hypaque density centrifugation.

Clonogenic assays

CAF-C assays were performed over four weeks using pre-treated healthy or MDS MSC layers. One thousand magnetically isolated CD34+ cells were added in StemMACS™ HSC-CFU complete medium (Miltenyi Biotec).

CFU assays were carried out using cells harvested after one week of co-culture, with 300 cells being plated in enriched methylcellulose medium with recombinant cytokines (MethoCult™ H4435, STEMCELL Technologies). Colonies were counted after 2 weeks and classified under a microscope or with the STEMvision™ system (STEMCELL Technologies).

MSC differentiation assays

Adipogenic and osteogenic differentiation capacity is an important functional feature of MSCs and often altered in MDS leading to changes in the bone metabolism. Therefore, the effect of tasquinimod on adipogenic and osteogenic differentiation was assayed as follows.

MSCs were seeded in 6-well plates (5×10^3 MSCs/cm²), cultured to subconfluency for approximately 4 days in DMEM, and subjected to adipogenic (0.5 mM 1-methyl-3-butyloxanthine, 1 µM dexamethasone, 100 µM indomethacin, 10 µM insulin) or osteogenic (0.1 µM dexamethasone, 0.2 mM ascorbate-2-phosphate, 10 mM β-glycerophosphate) differentiation. Adipogenesis was assessed by Oil Red O staining after 21 days.

Differentiation was graded according to microscopic analysis as follows: 0 = no differentiation; 1 = 25% of the culture wells contain differentiated cells; 2 = 50% of the culture wells contain differentiated cells; 3 = 75% of the culture wells contain differentiated cells; 4 = 100% of the culture wells contain differentiated cells. Osteogenesis was determined by van Kossa staining, and the catalytic activity of ALP was determined.

Detection of reactive oxygen species (ROS) levels

The inflammatory micromilieu of the myelodysplastic bone marrow leads to increased ROS levels which may cause aberrant cellular function. Therefore, the effect of tasquinimod on ROS levels in MSCs is assayed using the CellROX™ Deep Red Flow Cytometry Assay kit (Thermo Fisher) according to the manufacturer's instructions.

Seahorse Metabolic Extracellular Flux Profiling

The impact of tasquinimod on cellular metabolism is investigated by using the Seahorse system (Agilent Technologies). MSCs are seeded in Seahorse 96-well plates. Cell Mito Stress Test (XF Cell Mito Stress Test Kit, Agilent Technologies) is performed following the standard protocol. Oxygen consumption rate (OCR) and Extracellular Acidification Rate (ECAR) are detected after injection of oligomycin (1 mM), carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP, 0.5 mM), and the combination of rotenone and antimycin (Rot/AA, 0.5 mM). OCR is measured with an XF96 analyzer and the Wave software (version 2.2.0).

Western blot

Whole cell lysates were prepared using RIPA lysis buffer containing proteinase inhibitors. Protein concentrations were determined using the BCA Protein Assay Kit. Equal protein amounts were separated by SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes for detection of specific proteins using primary antibodies for IRAK1, NF- κ B-p65, gasdermin and OXPHOS overnight at 4°C, following secondary antibodies HRP-conjugated goat anti-mouse IgG (Invitrogen) or donkey anti-rabbit IgG (GE Healthcare), respectively. The blots were incubated with ECL Plus Western Blotting reagent (Amersham) and the signals captured using a LAS3000 imaging system.

RT-PCR

RNA was isolated from MSC using RNeasy Mini Kit (Qiagen) and reverse transcribed into cDNA using RevertAid™ cDNA synthesis kit (Thermo Fisher) with oligo-dT primers. Relative target quantity was determined using the comparative CT ($\Delta\Delta$ CT) method. RT-PCR was performed using SYBR Green/ROX PCR master mix (Thermo) and target specific primers for caspase1, IL1 β , IL18, PD-L1, PD-1 and GAPDH as housekeeping gene on a Taqman™ Fast 3500 cycler (Applied Biosystems). Amplicons were normalized to endogenous GAPDH control.

Immunohistochemistry

Bone marrow tissues of MDS patients and healthy donors were characterized by multiplex

immunohistochemistry. With this method the sequence and spatial distribution of CD271+ MSCs, CD68+ macrophages and CD66b+ neutrophils were determined in one staining and analyzed with the VECTRA® imaging system.

5 *Immunofluorescence staining and confocal laser scanning microscopy*

S100A9/tasquinimod treated MSCs were fixed with 4% paraformaldehyde. Cells were permeabilized using PBS containing 0.1% Triton™ X-100 (T-PBS), blocked with T-PBS containing 10% FCS and 1% HSA (IF-Buffer) and incubated over night at 4°C with antibodies against α SMA, NF κ B or PD-L1. Secondary antibodies polyclonal sheep-anti-rabbit-Cy3 or
10 polyclonal goat-anti-mouse-Cy2 were incubated for 1 h at room temperature. Cell nuclei were counterstained with DAPI. Imaging analysis was performed by confocal microscopy (LSM800, Carl Zeiss) and ZEN software.

Isolation and characterization of extracellular vesicles (EVs) from tasquinimod treated MSCs

15 EVs play an important role for the intercellular communication. These small membrane-surrounded particles carry different bioactive substances such as small RNA, mRNA and proteins. They are derived from the endosomal compartment or bud directly from the cell membrane. To test an influence of S100A9 and tasquinimod on MSC-derived EVs, they are isolated from serum-free culture supernatants by using the ExoEasy Maxi kit (Qiagen).
20 Concentration and size of the EVs is detected by Nanoparticle Tracking Analysis (NTA) using a ZetaView® instrument. The miRNA is isolated by the miRNeasy kit (Qiagen) and Taqman assays are performed with specific primers, e.g. for miR-145 and miR-146a. The functional impact of S100A9/tasquinimod-primed MSC-derived EVs is analyzed by incubation with PBMCs or HSPCs and subsequent flow cytometry.

25 Results

Higher S100A9 levels were detected in BM plasma of MDS patients compared to healthy controls, with the highest levels measured in the low-risk MDS (Figure 1).

30 *In vitro*, relevant S100A9 mRNA expression could not be detected in cultured MDS or healthy MSCs, which suggests that this cell type does not represent the main S100A9 source in the bone marrow microenvironment. Rather, staining of BM tissue demonstrated S100A8 and S100A9 expression mainly by CD66b+ neutrophils and with less extent by CD68+ macrophages.

35 Treatment of MDS MSCs with S100A9 induced TLR4 downstream signalling, as demonstrated by increased expression of IRAK1 and NF- κ B-p65. Additionally, higher

expression of gasdermin was detected; gasdermin is an inducer of pyroptosis in S100A9 treated cells. Addition of tasquinimod inhibited the expression of the mentioned proteins, indicating an alleviation of the induced inflammation (Figure 2).

- 5 The mRNA expression of proinflammatory cytokines such as IL-1 β and IL-18 as well as their activator caspase 1 increased in the MSCs after treatment with S100A9, and this increase was inhibited in the presence of tasquinimod (Figure 3).

PD-L1 as a potential downstream target that has the potential to inhibit efficient
10 hematopoiesis in MDS, was induced by S100A9 and could be suppressed by tasquinimod both at the mRNA and protein level. It was noted that the basic expression of PD-L1 was clearly higher in CMML than in healthy MSCs (Figure 4). Surprisingly, the downregulation of PD-L1 was noted in MSC from healthy volunteers and CMML MDS patients alike when tasquinimod even in the absence of S100A9. This is surprising also since previously,
15 tasquinimod has been reported to upregulate expression of PD-L1 (Oncoimmunology 2016, Vol. 5, No. 6, e1145333).

S100A9 treatment increased the expression of α SMA in MSCs which is typical for the differentiation of MSCs into myelofibroblasts present in the tumor microenvironment. In
20 parallel, an increased nuclear expression of NF- κ B was observed. Tasquinimod treatment reduces both α SMA and NF- κ B expression.

Adipogenic differentiation is often pathologically increased in MDS MSCs. The addition of tasquinimod decreased the number of adipocytes by 25-50% after 14 days of cultivation in
25 differentiation medium detected by Oil red O positive cells.

Addition of TNF- α to MSC cultures resulted in increased expression of IRAK1, NF- κ B-p65 and gasdermin. Again, addition of tasquinimod inhibited the expression of these protein (Figure 5). This, too, indicates that the effect of tasquinimod in MSCs may not be related to
30 S100A9.

Since functional alterations of MSCs induced by inflammasome activation have a significant impact on MDS progression, the potential of tasquinimod treatment of stromal cells to alter their subsequent support of normal hematopoiesis was investigated. To this end, MSC/HSPC
35 *in vitro* co-cultures were used in which the stromal layers were pre-treated with combinations of S100A9 and tasquinimod. Clearly, treatment with S100A9 decreased the number of CAF-Cs as well as the number of CFUs in a subsequent clonogenic assay indicating a disturbed

support of hematopoiesis by MSCs. Both the number of CAF-Cs and CFUs could be increased by tasquinimod (Figure 6a-c).

The PD-1 (receptor for PD-L1) expression in co-cultured HSPCs was regulated in the same way as its ligand in treated MSCs.

From the above findings it appears that the pathological inflammatory activation in the myelodysplastic bone marrow can be rescued by tasquinimod, by inhibition of NF- κ B-p65 signalling and PD-L1/PD-1 expression in MSC. These effects result in an improved hematopoietic support by MSCs, and thereby to an improvement of anemia and cytopenia in MDS patients.

Finally, the results of the MSC differentiation assays show that tasquinimod is capable of reducing adipogenic differentiation and of improving osteogenic differentiation. The results of the CellROX™ Deep Red Flow Cytometry Assay show that tasquinimod can block elevated ROS levels in MSCs. The results of the Seahorse Metabolic Extracellular Flux Profiling show that the effect of tasquinimod leads to reduced cellular stress and improved Spare Respiratory Capacity which is a readout for the cellular metabolic fitness.

In vivo validation of in vitro results

The promising *in vitro* results are validated *in vivo* in different mouse models. In these models, genetically modified mice (e.g. B6;129-*Trp53bp1^{tm1Jc}*/J and NUP98/HOXD13) which develop typical signs of MDS such as anemia and cytopenia are treated with tasquinimod. Peripheral blood counts are recorded weekly and finally the bone marrow is analysed after 20 weeks. These experiments show that tasquinimod is able to partly restore functional hematopoiesis.

Moreover, immunodeficient NSG mice are transplanted with HSPCs which have been co-cultured with pre-treated MSCs as described above to analyse a potential improved engraftment of cells derived from tasquinimod pre-treated cocultures. Peripheral blood of the recipients are obtained every 3–4 weeks by venipuncture of the retro-orbital venous plexus and the amount of human cells is analysed by flow cytometry after staining with antibodies against human CD45 and CD34. The final bone marrow analysis is performed after 20 weeks.

CLAIMS

1. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use in the treatment of myelodysplastic syndrome (MDS) in a mammal patient.
- 5 2. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 1, wherein the treatment is by oral administration.
3. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 1 or 2, wherein the treatment is by administration of an amount of from 0.001 mg to 0.2 mg of
10 tasquinimod/kg of body weight per day, or of a corresponding amount of the pharmaceutically acceptable salt thereof.
4. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to any one of claims 1 to 3, wherein the treatment is by administration of tasquinimod or the
15 pharmaceutically acceptable salt thereof, 1-3 times a day.
5. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to any one of claims 1 to 4, wherein tasquinimod or the pharmaceutically acceptable salt thereof is administered in a solid dosage form.
20
6. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 5, wherein the solid dosage form is a capsule, a tablet or a pill.
7. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to any one
25 of claims 1 to 4, wherein tasquinimod or a pharmaceutically acceptable salt thereof is administered dissolved or suspended in a liquid vehicle.
8. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to any one of claims 1 to 7, wherein the treatment further comprises radiation therapy and/or autologous
30 stem cell transplantation.
9. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to any one of claims 1 to 8, wherein the MDS is selected from MDS with unilineage (single lineage) dysplasia, MDS with multilineage dysplasia, MDS with ring sideroblasts (MDS-RS), MDS
35 associated with isolated del chromosome abnormality, such as MDS with isolated del(5q), MDS with excess blasts, and MDS, unclassifiable.

10. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 9, wherein the MDS is MDS with unilineage (single lineage) dysplasia.

11. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 9, wherein the MDS is MDS with multilineage dysplasia.

12. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 9, wherein the MDS is MDS with ring sideroblasts (MDS-RS).

13. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 9, wherein the MDS is MDS associated with isolated del chromosome abnormality.

14. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 9, wherein the MDS is MDS with excess blasts.

15. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 9, wherein the MDS is MDS, unclassifiable.

16. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to any one of claims 1 to 15, wherein the MDS is a very low risk MDS, low risk MDS, intermediate risk MDS, high risk MDS or very high risk MDS.

17. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 16, wherein the MDS is a very low risk MDS.

18. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 16, wherein the MDS is a low risk MDS.

19. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 16, wherein the MDS is an intermediate risk MDS.

20. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 16, wherein the MDS is a high risk MDS.

21. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 16, wherein the MDS is a very high risk MDS.

22. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to claim 1, wherein the MDS includes chronic myelomonocytic leukemia.

23. Tasquinimod, or a pharmaceutically acceptable salt thereof, for use according to any one of claims 1 to 22, for the improvement of one or more hematologic parameters in the mammal patient, the improvement comprising decreasing myoblasts, increasing hemoglobin, increasing platelets, increasing neutrophils, decreasing hepcidin, reducing units of red blood cell transfused, reducing frequency of transfusion, and/or reducing transfusion dependence.

24. Use of tasquinimod, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for the treatment of myelodysplastic syndrome (MDS).

25. The use according to claim 24, wherein the medicament is suitable for oral administration.

26. The use according to claim 24 or 25, wherein the medicament is suitable for administration of an amount of from 0.001 mg to 0.2 mg of tasquinimod/kg of body weight per day, or of a corresponding amount of the pharmaceutically acceptable salt thereof.

27. The use according to any one of claims 24 to 26, wherein the medicament is suitable for administration 1-3 times a day.

28. The use according to any one of claims 24 to 27, wherein the medicament is a solid dosage form.

29. The use according to claim 28, wherein the solid dosage form is a capsule, a tablet or a pill.

30. The use according to any one of claims 24 to 27, wherein the medicament contains tasquinimod or a pharmaceutically acceptable salt thereof dissolved or suspended in a liquid vehicle.

31. The use according to any one of claims 24 to 30, wherein the MDS is selected from MDS with unilineage (single lineage) dysplasia, MDS with multilineage dysplasia, MDS with ring sideroblasts (MDS-RS), MDS associated with isolated del chromosome abnormality, such as MDS with isolated del(5q), MDS with excess blasts, and MDS, unclassifiable.

32. The use according to any one of claims 24 to 31, wherein the MDS is a very low risk MDS, low risk MDS, intermediate risk MDS, high risk MDS or very high risk MDS.

33. The use according to claim 24, wherein the MDS includes chronic myelomonocytic leukemia.

34. The use according to any one of claims 24 to 33, wherein the medicament is for the improvement of one or more hematologic parameters in the mammal patient, the improvement comprising decreasing myoblasts, increasing hemoglobin, increasing platelets, increasing neutrophils, decreasing hepcidin, reducing units of red blood cell transfused, reducing frequency of transfusion, and/or reducing transfusion dependence.

35. A method for the treatment of myelodysplastic syndrome (MDS) in a mammal in need of such treatment, by administration of a therapeutically effective amount of tasquinimod or a pharmaceutically acceptable salt thereof to the mammal.

36. The method according to claim 35, wherein the treatment is by oral administration.

37. The method according to claim 35 or 36, wherein the treatment is by administration of an amount of from 0.001 mg to 0.2 mg of tasquinimod/kg of body weight per day, or of a corresponding amount of the pharmaceutically acceptable salt thereof.

38. The method according to any one of claims 35 to 37, wherein the treatment is by administration of tasquinimod or the pharmaceutically acceptable salt thereof, 1-3 times a day.

39. The method according to any one of claims 35 to 38, wherein tasquinimod or the pharmaceutically acceptable salt thereof is administered in a solid dosage form.

40. The method according to claim 39, wherein the solid dosage form is a capsule, a tablet or a pill.

41. The method according to any one of claims 35 to 38, wherein tasquinimod or a pharmaceutically acceptable salt thereof is administered dissolved or suspended in a liquid vehicle.

42. The method according to any one of claims 35 to 41, wherein the treatment further comprises radiation therapy and/or autologous stem cell transplantation.

43. The method according to any one of claims 35 to 42, wherein the MDS is selected from MDS with unilineage (single lineage) dysplasia, MDS with multilineage dysplasia, MDS with ring sideroblasts (MDS-RS), MDS associated with isolated del chromosome abnormality, such as MDS with isolated del(5q), MDS with excess blasts, and MDS, unclassifiable.

44. The method according to any one of claims 35 to 43, wherein the MDS is a very low risk MDS, low risk MDS, intermediate risk MDS, high risk MDS or very high risk MDS.

45. The method according to claim 35, wherein the MDS includes chronic myelomonocytic leukemia.

46. The method according to any one of claims 35 to 45, for the improvement of one or more hematologic parameters in the mammal patient, the improvement comprising decreasing myoblasts, increasing hemoglobin, increasing platelets, increasing neutrophils, decreasing hepcidin, reducing units of red blood cell transfused, reducing frequency of transfusion, and/or reducing transfusion dependence.

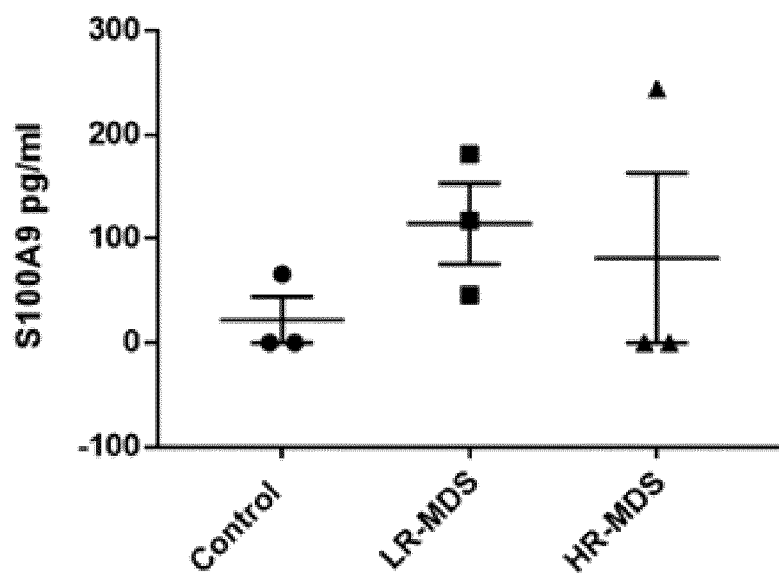


Figure 1

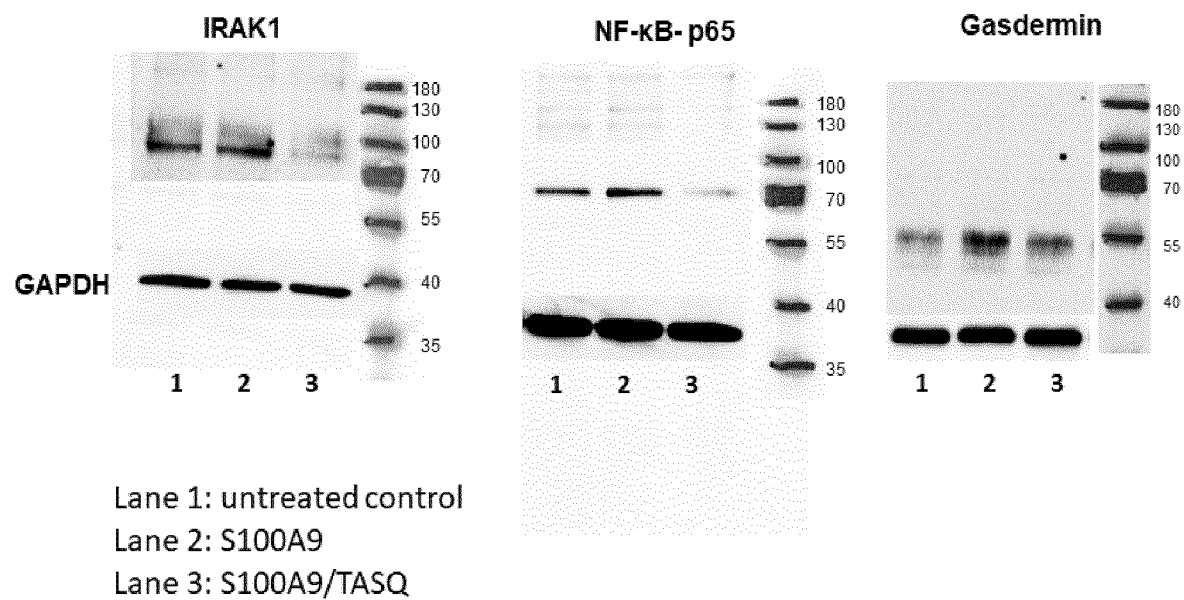


Figure 2

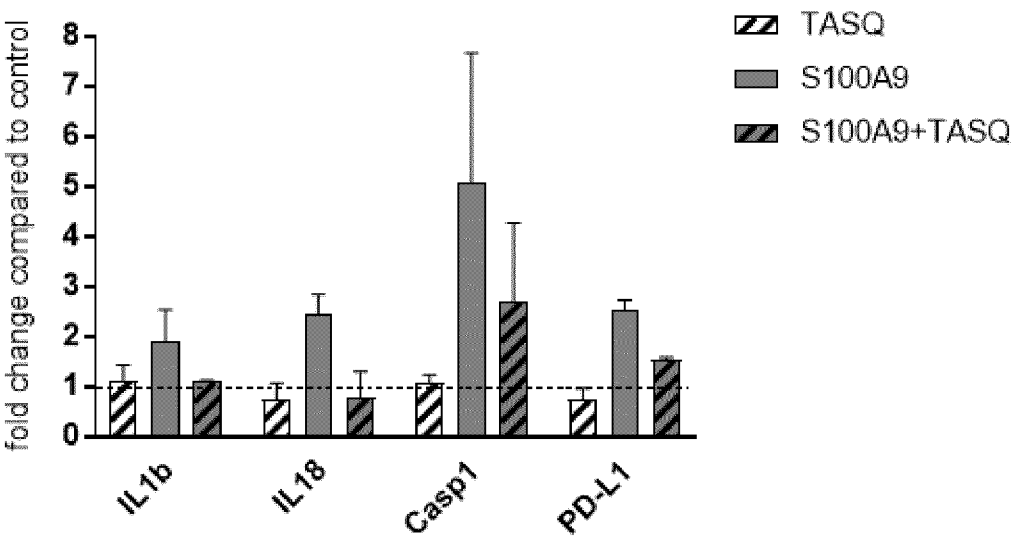


Figure 3

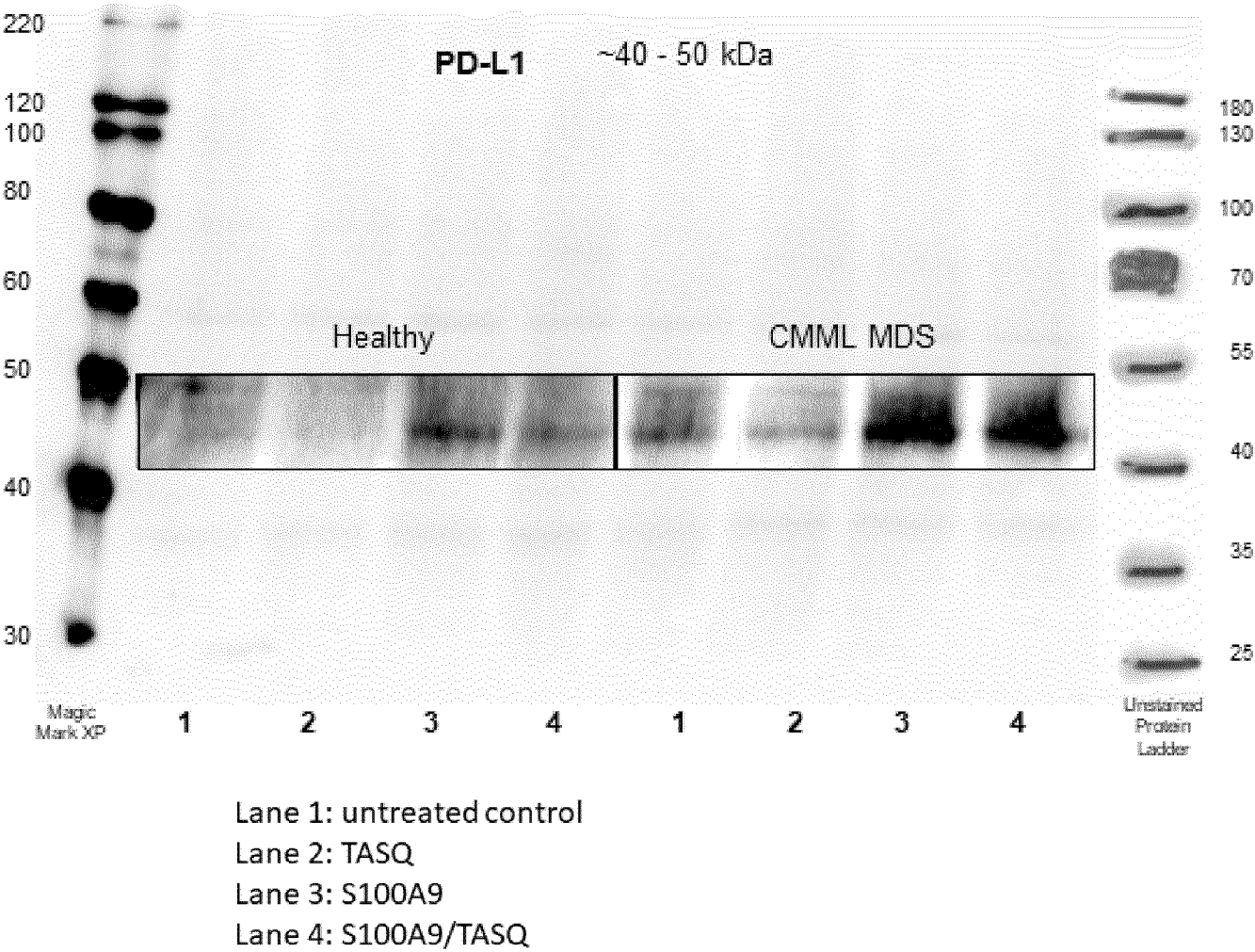


Figure 4

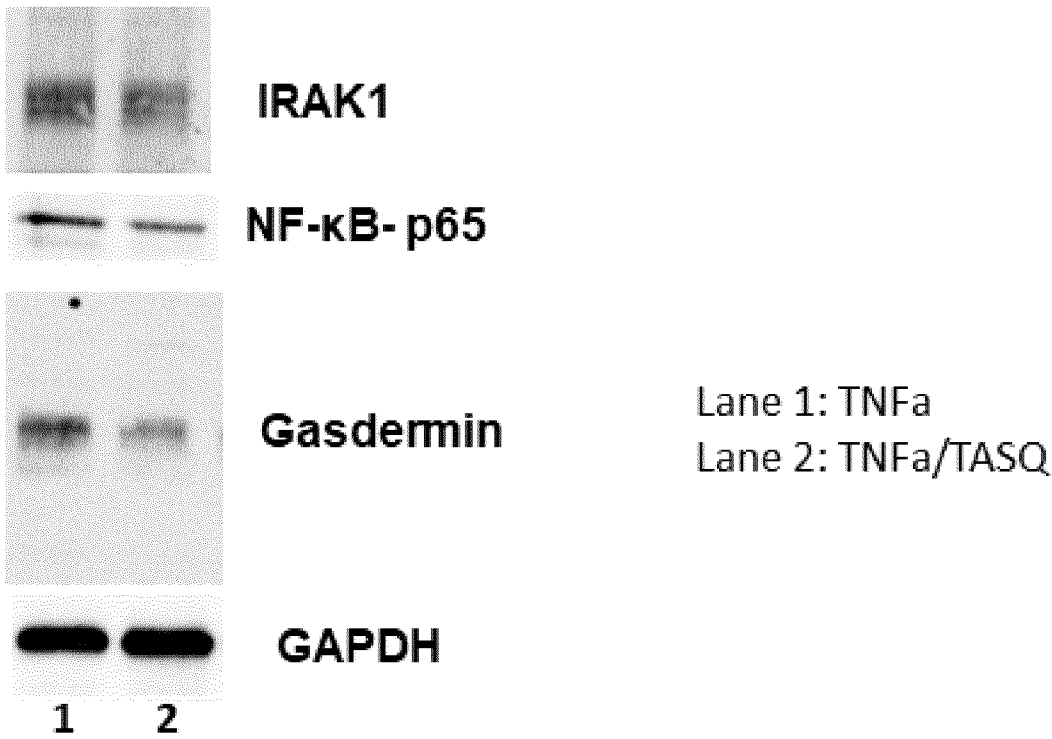


Figure 5

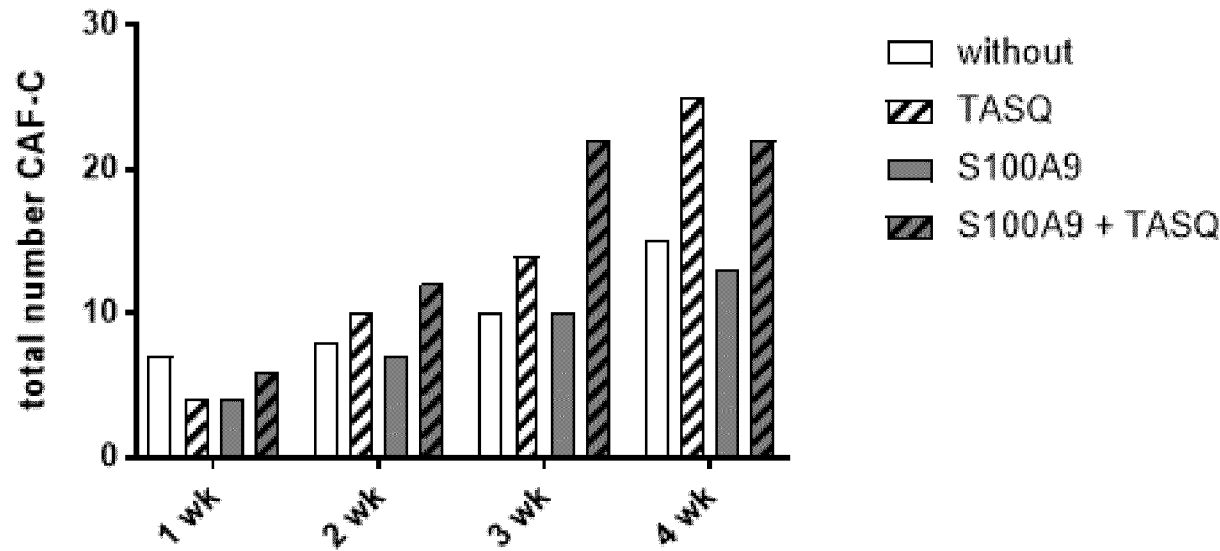


Figure 6a

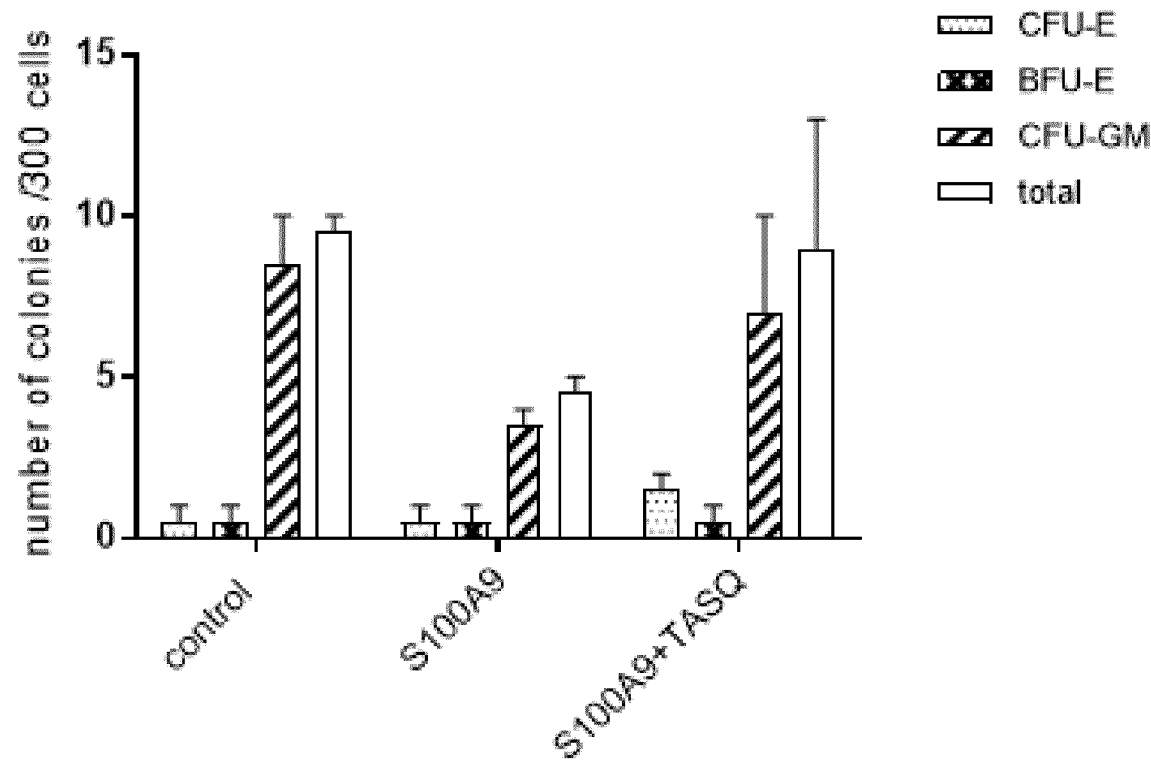


Figure 6b

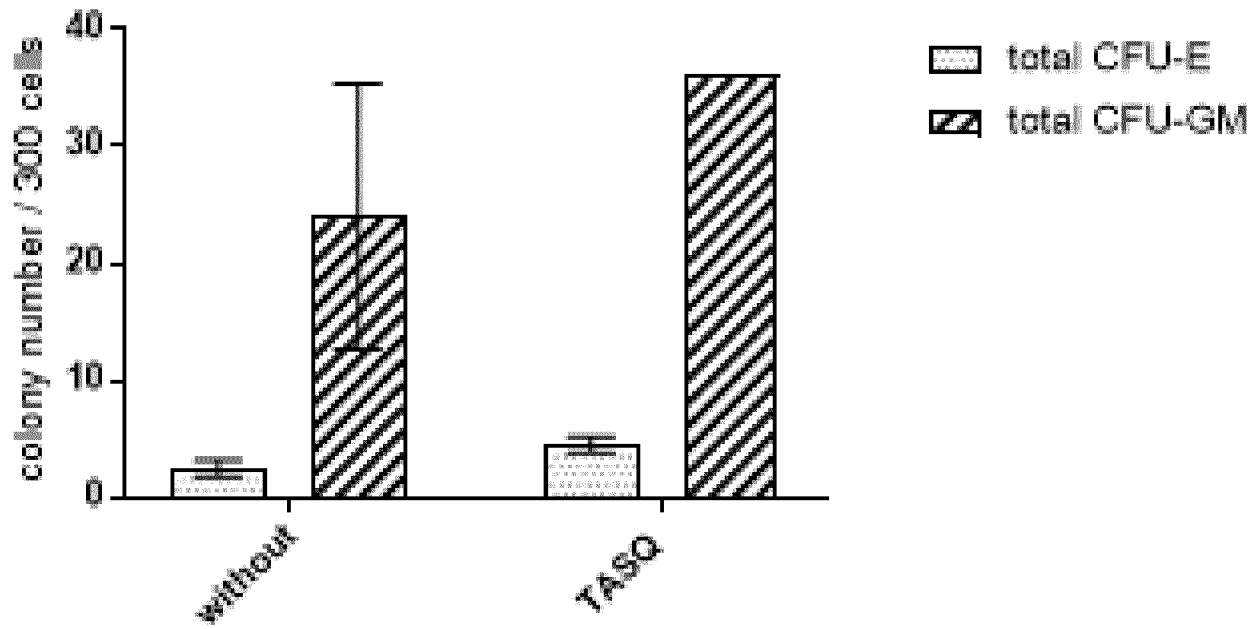


Figure 6c

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/050891

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K31/4704 A61K35/00 A61N5/00 A61K9/00 A61P35/00 A61P35/02 ADD. According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2004/035064 A1 (CELGENE CORP [US]) 29 April 2004 (2004-04-29) abstract page 7, line 15 - line 32 page 10, line 32 - page 17, line 1 examples 1-3 claims 1-36 -----	1-46
Y	WO 2016/078921 A1 (ACTIVE BIOTECH AB [SE]) 26 May 2016 (2016-05-26) cited in the application abstract page 1, line 4 - line 8 page 2, line 1 - line 6 page 5, line 1 - page 6, line 8 examples 1,2 claims 1-17 ----- -/--	1-46
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 31 March 2022		Date of mailing of the international search report 08/04/2022
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Taylor, Mark

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2022/050891

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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T	Anonymous: "Myelodysplastic Syndromes Treatment (PDQ)-Health Professional Version - National Cancer Institute", / 17 June 2021 (2021-06-17), XP055907558, Retrieved from the Internet: URL:https://www.cancer.gov/types/myeloproliferative/hp/myelodysplastic-treatment-pdq [retrieved on 2022-03-31] abstract Prognosis -----	1-46

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